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out over the reefs to the northward the color index was 3.9 (8.6 per cent. yellow), that of the ocean beyond the reefs about 3.5 (7 per cent. yellow).

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A SIMPLE METHOD OF MEASURING THE RATE OF RESPIRATION OF SMALL ORGANISMS

In view of the widespread interest at the present time in the subject of respiration in the lower organisms, it is thought that the following simple method of measuring the rate of carbon dioxide production in small non-aquatic animals and plants may be found useful not only by teachers who desire a quantitative method suitable for class instruction but also by investigators who wish, without any material sacrifice in accuracy, considerably to simplify the various procedures at present followed in making determinations of small amount of CO_2. The apparatus required may readily be constructed by anyone in a few minutes out of materials easily obtainable, and with it, it is possible to measure, with a probable error well within the normal uncontrollable range of variation of the material likely to be studied, a few thousandths of a milligram of carbon dioxide—an amount equal to that given out at ordinary temperatures by a sprouting grain of wheat in perhaps three or four minutes and by a house-fly in one or two minutes. It would be relatively easy still further to increase the delicacy of the method, though the gain in sensitiveness would be at the expense of the simplicity which in its present form is its chief recommendation.

The method is based upon the well known indicator methods of Haas[1] and of Osterhout,[2] but unlike the first, it is applicable to small non-aquatic organisms, and unlike the second, it involves the use of apparatus so simple in construction that it can be duplicated any desired number of times and can therefore be used even by large classes of elementary students. Furthermore, provision is made not only for the comparison of relative rates of carbon dioxide production but also for the measurement of absolute amounts. Simplicity is secured by taking advantage of two well-known facts: (1) that the carbon dioxide content of out-of-door air varies only slightly from day to day, and scarcely at all during the course of an ordinary series of experiments and (2) that the distribution of a quantity of carbon dioxide between given amounts of water and air may readily be calculated from the known absorption coefficients for this gas at various temperatures, which may be obtained from the *Landolt-Börnstein Tabellen* or elsewhere. The first fact obviates the necessity of removing all of the carbon dioxide from the apparatus at the beginning of the experiment and the second makes it possible, with a minimum of trouble, to prepare standards for comparison which contain any desired amount of carbon dioxide, thus enabling measurements of quantity as well as of rate of production to be made.

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[1] Haas, A. R., *Science*, 1916, XLIV, 105.

[2] Osterhout, W. J. V., *J. Gen. Physiology*, 1918, I, 17.

The apparatus in its simplest form consists of a Nonsol test tube about 75 mm. by 10 mm., which has been drawn out in the Bunsen flame somewhat above its middle into a constriction approximately 30 mm. long and 4 mm. in diameter. It is closed with a well-rolled cork which has been thoroughly soaked in, and coated with, acid free paraffin. The indicator solution is placed in the lower portion of the tube; the constriction prevents it, when the tube is agitated, from splashing on the organism contained in the upper portion. To enable quantitative measurements to be made, a series of standard tubes is required in which known amounts of carbon dioxide have been added to the same indicator solution as that used in the apparatus just described. For the preparation of these standards the following device is employed. A Pyrex or Nonsol flask with a capacity of about 150 c.c. is fitted with a well rolled cork through which a hole is bored and one of the unaltered Nonsol test tubes forced in such a way that when the cork is in the flask the bottom end of the tube projects freely upwards and its lip fits against the small end of the cork. After being thus prepared, the sides and the lower surface of the cork are thoroughly coated with paraffin of the best quality, partly to prevent leaks but chiefly to protect the indicator solution from actual contact with the cork, which would be very likely to cause changes in its color. It is desirable, though not absolutely necessary, to have as many of the flasks with the prepared stoppers as the number of standards to be employed—usually three to five. The only additional pieces of apparatus required are a carbon dioxide generator, a box for comparing the colors of the indicator tubes such as is commonly used in colorimetric methods of determining hydrogen ion concentration (a convenient form is supplied by the Hynson, Westcott and Dunning Co. of Baltimore, but it is easy to improvise one out of materials in hand in any laboratory) a fine-pointed pipette, a medium-sized test tube, a large flask, and a few pieces of glass tubing. For the pipette, tubing, etc., Pyrex or Nonsol glass should preferably be used; if ordinary glass be employed it should be coated with paraffin where it comes in contact with the solutions.

The first step in making a determination is the preparation of an indicator solution which is in exact equilibrium with the out-of-door air. This may conveniently be done as follows: A two-liter flask is filled with tap water, taken to an open window or out of doors and all of the water except about 100 c.c. is slowly poured out, great care being taken that neither the breath of the operator nor any currents of air from the laboratory come near it at this time. Enough of a concentrated solution of the indicator (phenolsulphonephthalein) is added to give a color of the proper intensity, the flask is stoppered and vigorously shaken for several minutes. If the tap water is not nearly in equilibrium with the carbon dioxide of the air, as shown by any decided change in color on shaking, the solution should be poured into a second flask from which water previously brought more nearly into equilibrium with the air has been emptied, and shaken for several minutes more. The solution, when in equilibrium with air at 16° C. (where the absorbtion coefficient is approximately equal to unity), and at a pressure of 760 mm. of mercury, contains approximately 0.3 c.c. of CO_2 per liter, or 0.0006 mg. per c.c. The exact amount need not be determined, however, since it is a constant quantity in all of the tubes used, and it is the amount *added to* it which is significant. To secure the benefit of the most sensitive part of the range of the indicator, the solution thus prepared should have a p H of approximately 7.6 to 7.8, *i.e.*, it should have a decided pink color with very little trace of orange. If the tap water is not alkaline enough to produce this result, a few drops of very weak NaOH may be added before the final shaking. If the tap water is too alkaline, it may be diluted with distilled water. The use of tap water rather than a weak solution of NaOH in distilled water is recommended merely for the sake of convenience and economy in those cases where it is suitable.

The next step is the preparation of the comparison tubes in which the indicator solutions, instead of being allowed to remain in equilibrium with ordinary air and therefore to contain at 16° C. 0.0006 mg. of CO_2 per c.c., has received additions in the various tubes of known amounts of this gas. This is accomplished as follows: A current of pure CO_2 from the generator is allowed to pass through a few c.c. of distilled water in the medium sized test tube until the water is saturated at atmospheric pressure. It is convenient to use in the test tube a stopper with two openings, one for the inlet tube which carries the gas below the surface of the water and the other for an open glass tube projecting from the upper part of the tube through the cork and several inches into the air. This permits the excess gas to escape, and since CO_2 is heavier than air, the test tube soon becomes filled with a pure atmosphere of it, the outside air not readily entering through the long and narrow escape tube. It is necessary that the current of CO_2 shall be slow enough to give no more than atmospheric pressure in the test tube. If only a few c.c. of water at a time are charged, the added pressure of 2 or 3 cm. of water, due to the dipping of the inlet tube below its surface, is not significant. The minimum time required to saturate the water has not been determined, but it is the custom of the writer when fresh water is taken to allow the current to flow for at least thirty minutes; afterwards, by keeping the tube corked between experiments, the water remains almost saturated and exact equilibrium may easily be established in five or ten minutes. It is, of course, very important that neither the test tube nor the tube admitting the CO_2 shall give off appreciable amounts of alkali, hence the recommendation that Pyrex or Nonsol glass be used or the same result be secured with inferior glass by means of a thin coating of paraffin.

Having a solution whose CO_2 content can accurately be calculated if the temperature and the barometric pressure are known, the next step is to add measured amounts of it to successive portions of the indicator solution. This is done as follows. The first flask and the test tube in its stopper are filled with distilled water, which by shaking has been brought into equilibrium with out-of-door air, and emptied with the precautions already noted. Five c.c. of the prepared indicator solution are then added, the cork quickly inserted and the whole vigorously shaken. The color of the solution should not change if the proper precautions have been taken. The remaining flasks are then treated in the same way. By inverting them, the color of the indicator solution in their respective test tubes can be compared against a white background. It should be the same in all. The first flask is kept as a control, nothing being added. It is inverted, and the cork with its test tube containing the indicator solution removed and quickly stoppered with a paraffined cork. To the second flask one drop of the carbon dioxide saturated water is added, to the second two drops, to the third four, or any desired number, etc. The stopper in each case is quickly replaced and the whole apparatus shaken vigorously until the CO_2 has distributed itself between the solution and the air. The test tubes are then removed and corked as described above. The amount of CO_2 added to each c.c. of the solution may now readily be calculated by taking into account the absorbtion coefficient for the temperature in question and the relative amounts of water and air. The volume of a single drop of the added solution has, of course, previously been determined by counting the number of drops required to give a volume of, for example, 5 c.c. In adding the CO_2 it is convenient to use a fine-pointed pipette which can be inserted in the escape tube of the test tube in which the water has been charged. This makes it unnecessary to remove the stopper of the latter or otherwise to disturb it. Unless the pipette is first filled with CO_2 (which may, however, readily be done and which is to be recommended), a little of the gas will escape from the free surface of the liquid within the pipette. If only a few drops are used from the lower portion of the pipette, however, no error will result if one works quickly enough. To drop the solution into the flasks without allowing any appreciable amounts of CO_2 to escape into the air requires a little practice, but after a few trials the best method is discovered and the necessary skill acquired.

When the standards have been prepared, which requires only a few minutes at the most, everything is ready for an actual measurement. The procedure is as follows. After filling the prepared test tube with out-of-door air, 1 c.c. of the indicator solution is placed in its lower portion and the organism to be studied in its upper portion, either free or attached to the cork by a loop of thread. The tube is closed and agitated gently, either continuously or at intervals, to mix the air and the indicator solution thoroughly. It is neither desirable nor necessary to shake the tube vigorously; with a little practise it will be discovered that a very slight movement of the proper sort will keep the liquid filled with bubbles and the air in the whole apparatus in circulation. From time to time the color of the solution is compared with that of the standards. It is obvious that since the indicator solution is the same in each case and the starting point is the same, the same color indicates the same amount of CO_2 added. It is only necessary, from the relative volumes of water and air in the apparatus and the absorbtion coefficient under the conditions of the experiment, to calculate the total amount of CO_2 produced by the organism to the time of the observation. Since the absorption of the CO_2 by the solution lags a little behind its production, it is well not to consider the time from the starting point to the first tube; from the first to the second, however, and the second to the third, etc., this factor is approximately the same in each case and therefore does not appreciably affect the results.

As to the delicacy of the method, the indicator solution and the air in the tube, if the temperature is 16° C. and the absorption coefficient therefore equal to 1, each contain 0.0006 mg. of CO_2 per c.c. A ten per cent. increase causes a slight visible change in the color of the indicator solution, so a production of 0.0003 mg. in the 5 c.c. tube ought theoretically to be detectable. In practise, however, it is desirable to work with somewhat larger amounts, *e.g.*, 0.001 mg. or more. In measuring the time, it is well instead of trying to determine the point at which the two tubes exactly match to take the average between the last observation where the unknown tube is pinker and the first where it is yellower than the comparison tube. To meet a possible objection, it may be said that when the carbon dioxide has increased one hundred per cent., the oxygen in the tube has decreased only approximately 0.2 per cent.; consequently changes in the amounts of oxygen available for the organism during an ordinary experiment are hardly significant.

In conclusion, it may be stated that the method has been tested by comparing it with that of Lund[3] on the same organism (a firefly) and the differences obtained were only of the order of magnitude observed where two successive observations were made on the same individual by the latter method alone; that is, within the limits of uncontrollable normal variations of the species in question.

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[3] Lund, *Biol. Bull.*, 1919, XXXVI, 105.